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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/00 | A2

(11) International Publication Number: WO 00/61126

(43) International Publication Date: 19 October 2000 (19.10.00)

(21) International Application Number: PCT/GB00/01284

(22) International Filing Date: 6 April 2000 (06.04.00)

(30) Priority Data:
9908175.4 9 April 1999 (09.04.99) GB

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHOD OF TREATING NEUROLOGICAL DISORDERS

(57) Abstract

The present invention relates to a method of treating a neurological disorder comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

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METHOD OF TREATING NEUROLOGICAL DISORDERS

BACKGROUND OF THE INVENTION

Cerebral ischemia leads to a selective pattern of neuronal damage in animals and in humans. However, the exact mechanisms of damage have not, heretofore, been fully elucidated. Several mechanisms appear to be involved to some degree including activation of voltage-gated ion channels, excitotoxicity, circulating free radicals, and apoptosis. Boxer and Bigge, Drug Discovery Today, 2, 219-228 (1997); del Zoppo et al., Drugs, 54, 299-324 (1997). Early studies have also demonstrated that L-glutamate was neurotoxic, resulting in the proposed "excitotoxic" hypothesis of neuronal cell damage. Olney et al., Neurotoxicity of Excitatory Amino Acids, Raven Press, New York, 95-117.

The role of excitatory amino acids, such as glutamic acid and aspartic acid, as the predominant mediators of excitatory synaptic transmission in the central nervous system, has been well established. Watkins and Evans, *Ann. Rev. Pharmacol. Toxicol.*, **21**, 165 (1981); Monaghan, Bridges, and Cotman, *Ann. Rev. Pharmacol. Toxicol.*, **29**, 365 (1989); Watkins, Krogsgaard-Larsen, and Honore, *Trends. Pharmacol. Sci.*, **11**, 25 (1990). These amino acids function in synaptic transmission primarily through excitatory amino acid receptors. The excitatory amino acids also participate in a variety of other physiological processes such as motor control, respiration, cardiovascular regulation, sensory perception, and cognition.

Excitatory amino acid receptors are classified into two general types, ionotropic and metabotropic. Receptors that are directly coupled to the opening of cation channels in the cell membrane of the neurons are termed "ionotropic." This type of receptor has been subdivided into at least three subtypes, which are defined by the depolarizing actions of the selective antagonists *N*-methyl-D-aspartate (NMDA), α–amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid (KA). The second general type of receptor is the G-protein or second messenger-linked "metabotropic" excitatory amino acid receptor. This second type, when activated by the agonists, leads to enhanced phosphoinositide (PI) hydrolysis in the postsynaptic cell. The metabotropic

glutamate receptors are divided into multiple sub-types, including the group I sub-type (mGluR1 and mGluR5) and group II sub-type (mGluR2 and mGluR3). Both types of receptors appear not only to mediate normal synaptic transmission along excitatory pathways, but also participate in the modification of synaptic connections during development and changes in the efficiency of synaptic transmission throughout life. Schoepp, Bockaert, and Sladeczek, *Trends in Pharmacol. Sci.*, **11**, 508 (1990); McDonald and Johnson, *Brain Research Reviews*, **15**, 41 (1990).

The excessive or inappropriate stimulation of excitatory amino acid receptors leads to neuronal cell damage, or loss, by way of a mechanism known as excitotoxicity. This process has been suggested to mediate the medical consequences of neuronal degeneration and, thus, makes the abatement of this processes an important therapeutic goal. Excitotoxicity has been implicated in the pathophysiology of acute and chronic neurological conditions including neurodegenerative disorders such, stroke, cerebral ischemia, spinal cord trauma, head trauma, Alzheimer's Disease, Huntington's Chorea, amyotrophic lateral sclerosis, AIDS-induced dementia, perinatal hypoxia, cardiac arrest, hypoglycemic neuronal damage, ocular damage and retinopathy, idiopathic and drug-induced Parkinson's Disease, and cerebral deficits subsequent to cardiac bypass surgery. Other neurological conditions implicated with glutamate dysfunction are those involving neuromodulation such as muscular spasms, migraine headaches, urinary incontinence, psychosis, opiate tolerance and withdrawal, anxiety, emesis, brain edema, chronic pain, convulsions, and tardive dyskinesia. The use of a neuroprotective agent, such as an AMPA or NMDA receptor antagonist, is believed to be useful in treating these disorders and/or reducing the amount of neurological damage associated with these disorders. Excitatory amino acid antagonists are also useful as analgesic agents. United States Patents No. 4,902,695, United States Patents No. 5,670,516, United States Patents No. 5,356,902, and United States Patents No. 4,446,051 provides antagonists of AMPA and NMDA receptors useful for treating the aforementioned conditions. United States Patents Nos. 5,500,420 and 5,750,566 provide examples of mGluR agonists that are also useful for treating the aforementioned conditions.

Specifically, many studies have shown that AMPA receptor antagonists are neuroprotective in focal and global ischemia models. Gill, *Cerebrovascular and Brain Metab. Rev.*, **6**, 225 (1994); Gill and Lodge, *Neuropharmacology*, **33**, 1529 (1995); O'Neill *et al., Neuropharmacology* **37,** 1211 (1998). The competitive AMPA receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7 sulfamoylbenzo[*f*]quinoxaline) has been reported effective in preventing global and focal ischemic damage. (See Sheardown *et al., Science*, **247**, 571 (1990); Buchan *et al., Neuroreport*, **2**, 473 (1991); LePeillet *et al., Brain Research*, **571**, 115 (1992). The noncompetitive AMPA receptor antagonist GKYI 52466, a 2,3 benzodiazepine, has been shown to be an effective neuroprotective agent in rat global ischemia models. LaPeillet *et al., Brain Research*, **571**, 115 (1992). Likewise, substituted 2,3-benzodiazepine analogues of GYKI52466, namely LY300164 and LY300168, have also provided neuroprotection in gerbil global cerebral ischemia. Lodge *et al., Neuropharmacology,* **35,** 1681 1996.

Further, many studies have also shown that NMDA receptor antagonists are neuroprotective in animal models of global and focal cerebral ischemia. (See generally: Bullock and Fujisawa, *Journal of Neurotrauma*, **9** (supplement 2), S443 (1992); McCulloch, *Journal of Clinical Pharmacology*, **34**, 106 (1992);Park et al, *Neuroscience Letters*, **147**, 41 (1992); Scatton *et al.*, *Cerebrovascular Disease*, **1**, 121 (1991)) . For example, studies have shown that the competitive NMDA antagonist D-(–)CPP-ene provided protection in a focal cerebral ischemia model in cats, that the competitive NMDA antagonist CGS 19755 provided protection in a focal cerebral ischemia model in rats, and that the competitive NMDA antagonist LY233053 provided protection in a CNS ischemia model in rabbits. Bullock *et al.*, *Journal of Cerebral Blood Flow and Metabolism*, **10**, 668 (1990); Simon and Shirasho, *Annals of Neurology*, **27**, 606 (1990); Madden *et al.*, *Journal of Neurosurgery*, **76**, 106 (1992). The non-competitive NMDA antagonist dizocilpine provided protection in models of focal cerebral ischemia in cats and rats. Park *et al.*, *Journal of Cerebral Blood Flow and Metabolism*, **8**, 757 (1988); Park *et al.*, *Annals of Neurology*, **24**, 543 (1988). The competitive NMDA antagonist LY274614 is neuroprotective in an animal model of Huntington's Disease. Schoepp, *et al.*, *Journal of Neural Transmission [General*

Section], **85**, 131 (1991). Molecules that inhibit the glycine site (HA 966, ACEA 1021, L-701,324 and the like) and polyamine sites of the NMDA receptor complex (Ifenprodil, eliprodil, and the like) have also provided neuroprotection in model of global and focal cerebral ischaemia (Hicks *et al., Brain Res.* **819,** 65 (1999); Warner *et al., Journal of Cerebral Blood Flow and Metabolism* **15,** 188 (1995); Gotti *et al., J. Pharmacol. Exp. Ther.* **247,** 1211 (1988); Bath *et al., Eur. J. Pharmacol.* **299,** 103 (1996).

Other recent studies have demonstrated that kainate (in particular iGluR5) antagonists are neuroprotective in global (O'Neill *et al., Neuropharmacology* **37,** 1211 (1998) and focal (O'Neill et al., Neuropharmacology, 2000, in press) cerebral ischemia.

mGlu receptors are increasingly being considered as targets for the therapeutic intervention into neurodegenerative disorders, as their activation affects intracellular events which contribute both to the induction and progression of neuronal damage (Schoepp and Conn, *Trends Pharmacol Sci* **14,** 13 (1993); Nicoletti *et al., Trends Neurosci* **19,** 267 (1996); Buisson *et al, Eur J Neurosci* **8,** 138 (1996); Bruno *et al., J Neurosci* **18,** 9594 (1998)). The lack of selective agents has made it difficult to clarify the exact contribution of the various mGlu receptors to neurodegenerative diseases. However, recently, several new ligands for metabotropic glutamate receptors have been synthesised which have allowed the role of mGlu receptors in ischaemia to be studied in detail (Pin *et al., Eur J Pharmacol* **375,** 277 (1999); Schoepp *et al., Neuropharmacology* **38,** 1431 (1999)). It has recently been demonstrated that the newer group II mGluR agonists LY354740 and LY379268 are systemically active and provide neuroprotection in global cerebral ischemia (Bond *et al., NeuroReport* **9,** 1191 (1998); Bond *et al. Neurosci Lett* **273,**191 (1999). Likewise, antagonists of group I mGlu receptors have also been demonstrated to be neuroprotective. Thus, the selective mGluR1 antagonist, LY367385 (Bruno *et al., Neuropharmacology* **38,** 199 (1999) and the selective mGluR5 antagonist, MPEP (O'Neill *et al.,* 2000, submitted) protect against ischemia-induced cell death.

The role of nitric oxide in cerebral ischemia has also been investigated. In mice deficient in neuronal nitric oxide synthase (NOS), it has been demonstrated

that there is a reduction in infarct volume after middle cerebral artery occlusion (Huang et al., *Science*, **265**, 1883 (1994), and reduced hippocampal damage after global ischemia (Panahian et al., *Neuroscience*, **72**, 343 (1996). In contrast, endothelial nitric oxide synthase-knockout mice have larger infarcts after focal ischemia. Huang et al., *J. Cereb. Blood Flow Metab.*, **16**, 981 (1996). As a result, NOS inhibitors have been examined as possible neuroprotective agents. Earlier studies examined the effects of N^G-nitro-L-arginine methyl ester (L-NAME) in global and focal ischemia. Caldwell et al., *Eur. J. Pharmacol.*, **260**, 191 (1994); Dawson et al., *Cerebrovascular Brain Metab. Rev.*, **6**, 299 (1994). However, L-NAME is not selective for neuronal nitric oxide synthase over endothelial nitric oxide synthase. Recently, it has been reported that 7-nitroindazole is a specific inhibitor of neuronal NOS, which does not effect blood pressure and reduces infarct volume in focal ischemia. Yoshida et al., *J. Cereb. Blood Flow Metab.*, **14**, 924, (1994). It has also been demonstrated that 7-nitroindazole also protects against ischemic brain damage in the gerbil. O'Neill et al., *Eur. J. Pharmacol.*, **310**, 115 (1996). In another recent study, Zhang et al. reported that ARL 17477, a potent and selective neuronal NOS inhibitor, decreases infarct volume after transient middle cerebral artery occlusion in rat. *J. Cereb. Blood Flow Metab.*, **16**, 599 (1996). There is also evidence suggesting that apoptotic mechanisms also contribute to neuronal cell death in many disease states. Thompson, *Science*, **267**, **a1**, 1445 (1995); Thonberry and Lazebnik, *Science*, **281**, 1312 (1998). Therefore, caspase inhibitors or other anti-apoptotic strategies may also be beneficial in ischemic conditions.

To date, none of the aforementioned strategies for neuroprotection have had any clinical success and recent reviews have suggested certain combination therapies may be an alternative approach to the treatment of neurodegenerative disorders. Koroshetz and Moskowitz, *Trends In Pharmacol.*, **17**, 227 (1996); Boxer and Bigge, *Drug Discovery Today*, **2**, 219 (1997); Sacchetti *et al., Neurology*, **49**, S7-S74 (1997); and del Zoppo et al., *Drugs*, **54**, 299 (1997). Indeed, it has been reported that there is an added neuroprotective effect with dextrorphan and cyclohexamide in a rat model of focal cerebral ischemia (Du *et al., Brain Res.*, **718**, 233 (1996)) and other studies reported synergistic effects of

caspase inhibitors in combination with the NMDA inhibitor, MK-801, after transient focal cerebral ischaemia in mice. Ma et al., *Br. J. Pharmacol.*, **124**, 756 (1998). Interestingly, there is no disclosure of combining excitatory amino acid receptor antagonists with nitric oxide synthase inhibitors.

Surprisingly, we have discovered that combining an excitatory amino acid receptor antagonist, particularly an NMDA or AMPA receptor antagonist, with a nitric oxide synthase inhibitor produced synergistic effects in alleviating global cerebral ischemia. Thus, the present invention could address a long felt need for an effective treatment of neurological disorders, such as cerebral ischemia. The treatment of neurological disorders is hereby furthered.

SUMMARY OF THE INVENTION

The present invention provides a method of treating a neurological disorder comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

The present invention further provides a method of treating a neurodegenerative disease comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

More specifically, the present invention provides a method of preventing ischemia-induced cell damage such as may be caused by strokes, myocardial infarction, cardiac arrest or during transplantation, comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

In addition, the present invention provides a method of treating a neurological disorder or a neurodegenerative disease comprising administering to a patient an effective amount of a compound which possesses the combined activities of a nitric oxide synthase inhibitor and an excitatory amino receptor modulator.

In addition, the invention provides a pharmaceutical composition comprising a nitric oxide synthase inhibitor and an excitatory amino modulator, in

combination with one or more pharmaceutically acceptable carriers, diluents, or excipients.

The present invention further provides the use of an excitatory amino acid receptor modulator in combination with a nitric oxide synthase inhibitor for the manufacture of a medicament for treating a neurological disorder.

According to yet another aspect, the present invention provides a method of potentiating the neuroprotective effect of an excitatory amino acid receptor modulator in a patient, comprising administering to said patient an effective amount of a nitric oxide inhibitor.

The invention also provides a method of potentiating the neuroprotective effect of a nitric oxide inhibitor in a patient, comprising administering to said patient an effective amount of an excitatory amino acid receptor modulator.

The invention also provides the use of a nitric oxide synthase inhibitor for the manufacture of a medicament for potentiating the neuroprotective effect of an excitatory amino acid receptor modulator, and the use of an excitatory amino acid receptor modulator for the manufacture of a medicament for potentiating the neuroprotective effect of a nitric oxide synthase inhibitor.

DETAILED DESCRIPTION OF THE INVENTION

The term “excitatory amino acid receptor modulator” refers to an excitatory amino acid receptor antagonist or an excitatory amino acid receptor agonist.

The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds employed in the present invention which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a pharmaceutically acceptable mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts.

It will be understood by the skilled reader that most or all of the compounds used in the present invention are capable of forming salts, and that the salt forms of pharmaceuticals are commonly used, often because they are more readily crystallized and purified than are the free bases. In all cases, the use of the pharmaceuticals described herein as salts is contemplated in the

description herein, and often is preferred, and the pharmaceutically acceptable salts of all of the compounds are included in the names of them.

Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as *p*-toluenesulfonic, methanesulfonic acid, oxalic acid, *p*-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, hydrochloride, dihydrochloride, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, α-hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, napththalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

It should be recognized that the particular counterion forming a part of any salt of this invention is usually not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

As used herein the term “NOS” refers to nitric oxide synthase.

As used herein the term “neurological disorder” refers a disorder of the nervous system including but not limited to global and focal cerebral ischaemia, stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage as in cardiac arrest, neonatal distress, and the like.

As used herein the term “neurodegenerative disease” refers to Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and the like.

The neurodegenerative diseases are chronic conditions. The term "chronic" means a deteriorating condition of slow progress and long continuance. As such, a chronic neurodegenerating condition is treated when it is diagnosed and treatment continued throughout the course of the disease.

Ischemia represents a phenomenon in which tissue is deprived of either partial or total blood flow in conjunction with hypoxia. It may occur as an acute event or a chronic condition. The term "acute" means an exacerbated condition of short course followed by a period of remission. Thus, the treatment of ischemia induced cell damage contemplates both acute and chronic forms. In an acute event, compound is administered at the onset of symptoms and discontinued when the symptoms disappear. As described above, a chronic condition is treated throughout the course of the disease.

As used herein the term "patient" refers to a mammal, such a mouse, gerbil, guinea pig, rat, dog or human. It is understood that the preferred patient is a human.

It is understood that included within the term “excitatory amino acid receptor antagonist” are AMPA receptor antagonists, NMDA receptor antagonists, kainate receptor antagonists and metabotropic glutamate receptor antagonists. It is understood that any AMPA receptor antagonist or any NMDA receptor antagonist, as appreciated by one of ordinary skill in the art, are included within the scope of the present invention. Such AMPA receptor antagonists and NMDA receptor antagonists are readily available or are readily prepared by one of ordinary skill in the art following recognized procedures.

Examples of AMPA receptor antagonists include but are not limited to NBQX, GKYI 52466, the compounds disclosed in U.S. Patent No. 5,670,516, issued September 23, 1997, such as LY293558, U.S. Patent 5,446,051, issued

August 29, 1995, U.S. Patent 5,536,832, issued July 16, 1996, such as LY300164, and LY300168, the disclosures of which are incorporated herein by reference, and YM90-K, YM872, and the like.

Examples of NMDA receptor antagonists include but are not limited to MK-801, CNS1102 (Cerostat), Aptiganel, Ro-01-6794/706 (dextrorphan), Dextromethorphan, NPS1506, memantine (non-competitive NMDA antagonists); D-(–)CPP-ene, CGS 19755 (Selfotel), LY233053, LY202157, Ramacemide (competitive NMDA antagonists); HA-966, ACEA1021, GV150526A, L-701273 (glycine site NMDA antagonists); ifenprodil, eliprodil (polyamine site NMDA antagonists), and the like.

Examples of kainate receptor antagonists include but are not limited to LY377770, LY382884, and the like.

Examples of mGluR agonists include but are not limited to DCG-IV, ACPD, 4C3HPG, L-CCG-1, the compounds disclosed in U.S. Patent No. 5,500,420 such as LY354740, LY379268 and the like.

Examples of mGluR antagonists include but are not limited to MCPG, MPEP, LY367385, LY367366, LY393675 and the like.

Examples of nitric oxide synthase inhibitors include but are not limited to N^G-nitro-arginine, N^G-nitro-L-arginine methyl ester (L-NAME), 7-nitroindazole (7-NI), 3-Bromo-7-nitroindazole, L-MIN, 1-(2-trifluoromethylphenyl) imidazole TRIM), and ARL 17477, and the like.

It is further understood that the excitatory amino acid receptor modulators (including the AMPA receptor antagonists and the NMDA receptor antagonists), and the NOS inhibitors may exist as pharmaceutically acceptable salts, and that such salts are included within the scope of the present invention. It will also be appreciated that the excitatory amino acid receptor modulator may be generated *in vivo* by administering a pro-drug that is converted *in vivo* into the excitatory amino acid receptor modulator.

As used herein, the terms “treating” or “to treat” each mean to alleviate symptoms, eliminate the causation either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of the named disorder. As such, the methods of this invention encompass both therapeutic and prophylactic administration.

As used herein the term "effective amount" refers to the amount or dose of the compound, upon single or multiple dose administration to the patient, which provides the desired effect in the patient under diagnosis or treatment.

An effective amount can be readily determined by the attending diagnostician, as one skilled in the art, by the use of known techniques and by observing results obtained under analogous circumstances. In determining the effective amount or dose of compound administered, a number of factors are considered by the attending diagnostician, including, but not limited to: the species of mammal; its size, age, and general health; the specific neurological disorder involved; the degree of or involvement or the severity of the neurological disorder; the response of the individual patient; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances.

A typical daily dose will contain from about 0.01 mg/kg to about 100 mg/kg of each compound used in the present adjunctive therapy. Preferably, daily doses will be about 0.05 mg/kg to about 50 mg/kg, more preferably from about 0.1 mg/kg to about 25 mg/kg.

The adjunctive therapy of the present invention is carried out by administering a nitric oxide synthase inhibitor together with an excitatory amino modulator in any manner which provides effective levels of the particular compounds in the body at the same time. The nitric oxide synthase inhibitor and the excitatory amino modulator may be administered together, in a single dosage form, or may be administered separately. Oral administration is a preferred route.

However, oral administration is not the only route or even the only preferred route. One of the compounds may be administered by one route, such as oral, and the other may be administered by the transdermal, percutaneous, intravenous, intramuscular, intranasal or intrarectal route, in particular circumstances. The route of administration may be varied in any way, limited by the physical properties of the compounds and the convenience of the patient and the caregiver.

The adjunctive combination may be administered as a single pharmaceutical composition, and so pharmaceutical compositions incorporating both compounds are important embodiments of the present invention. Such compositions may take any physical form which is pharmaceutically acceptable, but orally usable pharmaceutical compositions are particularly preferred. Such adjunctive pharmaceutical compositions contain an effective amount of each of the compounds, which effective amount is related to the daily dose of the compounds to be administered. Each adjunctive dosage unit may contain the daily doses of all compounds, or may contain a fraction of the daily doses, such as one-third of the doses. Alternatively, each dosage unit may contain the entire dose of one of the compounds, and a fraction of the dose of the other compounds. In such case, the patient would daily take one of the combination dosage units, and one or more units containing only the other compounds. The amounts of each compound to be contained in each dosage unit depends on the identity of the compounds chosen for the therapy, and other factors such as the indication for which the adjunctive therapy is being given.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 mg to about 500 mg of each compound individually or in a single unit dosage form, more preferably about 5 mg to about 300 mg (for example 25 mg). The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for a patient, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient.

The inert ingredients and manner of formulation of the adjunctive pharmaceutical compositions are conventional, except for the presence of the combination of the present invention. The usual methods of formulation used in pharmaceutical science may be used here. All of the usual types of compositions may be used, including tablets, chewable tablets, capsules, solutions, parenteral solutions, intranasal sprays or powders, troches, suppositories, transdermal patches and suspensions. In general, compositions contain from about 0.5% to about 50% of the compounds in total, depending on the desired doses and the type of composition to be used. The amount of the

compounds, however, is best defined as the effective amount, that is, the amount of each compound which provides the desired dose to the patient in need of such treatment. The activity of the adjunctive combinations do not depend on the nature of the composition, so the compositions are chosen and formulated solely for convenience and economy. Any of the combinations may be formulated in any desired form of composition.

Capsules are prepared by mixing the compound with a suitable diluent and filling the proper amount of the mixture in capsules. The usual diluents include inert powdered substances such as starch of many different kinds, powdered cellulose, especially crystalline and microcrystalline cellulose, sugars such as fructose, mannitol and sucrose, grain flours and similar edible powders.

Tablets are prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as the compound. Typical diluents include, for example, various types of starch, lactose, mannitol, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Typical tablet binders are substances such as starch, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including acacia, alginates, methylcellulose, polyvinylpyrrolidine and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders.

Tablets are often coated with sugar as a flavor and sealant. The compounds may also be formulated as chewable tablets, by using large amounts of pleasant-tasting substances such as mannitol in the formulation, as is now well-established practice. Instantly dissolving tablet-like formulations are also now frequently used to assure that the patient consumes the dosage form, and to avoid the difficulty in swallowing solid objects that bothers some patients.

A lubricant is often necessary in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant is chosen from such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils.

Tablet disintegrators are substances which swell when wetted to break up the tablet and release the compound. They include starches, clays, celluloses,

algins and gums. More particularly, corn and potato starches, methylcellulose, agar, bentonite, wood cellulose, powdered natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp and carboxymethylcellulose, for example, may be used, as well as sodium lauryl sulfate.

Enteric formulations are often used to protect an active ingredient from the strongly acid contents of the stomach. Such formulations are created by coating a solid dosage form with a film of a polymer which is insoluble in acid environments, and soluble in basic environments. Exemplary films are cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methylcellulose phthalate and hydroxypropyl methylcellulose acetate succinate.

When it is desired to administer the combination as a suppository, the usual bases may be used. Cocoa butter is a traditional suppository base, which may be modified by addition of waxes to raise its melting point slightly. Water-miscible suppository bases comprising, particularly, polyethylene glycols of various molecular weights are in wide use, also.

Transdermal patches have become popular recently. Typically they comprise a resinous composition in which the drugs will dissolve, or partially dissolve, which is held in contact with the skin by a film which protects the composition. Many patents have appeared in the field recently. Other, more complicated patch compositions are also in use, particularly those having a membrane pierced with innumerable pores through which the drugs are pumped by osmotic action.

It is understood by one of ordinary skill in the art that the above procedures can also be applied to a method of treating a neurological disorder or a neurodegenerative disease comprising administering to a patient an effective amount of a compound which possesses the combined activities of a nitric oxide synthase inhibitor and an excitatory amino receptor modulator.

The following examples are provided in order to illustrate the method of use of the present invention. The reagents and starting materials are readily available to one of ordinary skill in the art. These examples are intended to be illustrative only and are not to be construed so as to limit the scope of the invention in any way. As used herein, the following terms have the meanings indicated: “i.p.” refers to intraperitoneally; "eq" or "equiv." refers to equivalents;

"g" refers to grams; "mg" refers to milligrams; "L" refers to liters; "mL" refers to milliliters; "μL" refers to microliters; "mol" refers to moles; "mmol" refers to millimoles; "psi" refers to pounds per square inch; "mm Hg" refers to millimeters of mercury; "min" refers to minutes; "h" or "hr" refers to hours; "°C" refers to degrees Celsius; "TLC" refers to thin layer chromatography; "HPLC" refers to high performance liquid chromatography; "R_f" refers to retention factor; "R_t" refers to retention time; "δ"refers to part per million down-field from tetramethylsilane; "THF" refers to tetrahydrofuran; "DMF" refers to N,N-dimethylformamide; "DMSO" refers to methyl sulfoxide; and "RT" refers to room temperature.

Male Mongolian gerbils (Bantin and Kingman, Hull, UK) at least 3 months old and weighing in excess of 60 g were used. The animals were maintained in standard lighting conditions and food and water were available ad libitum. The animals were anaesthetized with a 5% halothane/oxygen mixture and maintained using 2% halothane delivered with oxygen at 1l/min via a face mask throughout the procedure. Through a midline cervical incision, both common carotid arteries were exposed and freed from surrounding connective tissue. In animals to be rendered ischemic, both common carotid arteries were clamped for 5 min. At the end of the occlusion period blood flow was re-established. In sham-operated animals the arteries were exposed but not occluded. The wound was then sutured and the animals allowed to recover. Throughout surgery body temperature was maintained at 37°C using a "K-TEMP" temperature controller/heating pad (International Market Supply, Cheshire, U.K.) and brain temperatures were maintained using a heating lamp. After surgery the animals were placed in a four compartmental thermacage (Beta Medical and Scientific, U.K.) which maintained the environmental temperature at 28°C and rectal temperatures were measured for a 12 hour period after occlusion.

Histology

Five days after surgery the animals were perfused transcardially with 30 ml of 0.9% saline followed by 100 ml of 10% buffered formalin solution. The brains were removed and placed in 10% buffered formalin for 3 days, processed

and embedded in paraffin wax. 5 μm coronal sections were taken 1.5, 1.7 and 1.9 mm caudal to bregma using a microtome (Leitz 1400 sledge microtome). The sections were stained with haematoxylin and eosin and the neuronal density in the CA1 subfield of the hippocampus was measured using a microscope with grid lines (0.05 mm x 0.05 mm). The neuronal density is expressed as the number of viable cells per mm CA1 hippocampus. Statistical analysis of histological data was assessed using a 2-tailed unpaired Students *t*-test, with p values < 0.05 being considered statistically significant.

Experimental protocols

MK-801 (2.5 mg/kg i.p.) was administered 30 min prior to occlusion, LY293558 (20 mg/kg i.p.) was administered 30 min before occlusion reducing the dose to 10mg/kg for subsequent injections at 2hr 30 min and 5 hr 30 min after occlusion. ARL17477 (25mg/kg i.p.) and 7-nitroindazole (25mg/kg i.p.) were administered immediately and again 3hrs post occlusion. All treatments were given by the intraperitoneal route.

Results

5 μm sections taken 1.5 - 1.9 mm caudal to the bregma in the anterior hippocampus were examined under a microscope with grid lines. The CA1 pyramidal neurones were found to be degenerated in the 5 min occluded animals. The neuronal death involved nearly all the pyramidal neurones and this neurodegeneration was not obviously evident in any other forebrain region. The pyramidal cell density was counted at three different stereotaxic levels in the CA1 region of the hippocampus and the results expressed as mean $\pm$ S.E.M. neuronal density per 1 mm CA1.

In the first two experiments we evaluated the effects of MK-801 administered at 2.5 mg/kg i.p 30 min prior to occlusion alone or in combination with either 7-nitroindazole 25mg/kg i.p or ARL17477 25mg/kg i.p administered immediately and 3hrs post occlusion. The results indicated that MK-801 provided significant protection (18 and 26%) in both experiments. 7-nitroindazole provided some evidence of neuroprotection (10-18%), however this

result did not reach statistical significance. When dosed in combination, MK-801 and 7-Nitroindazole provided an enhanced (49%) neuroprotective effect. When dosed alone, ARL17477 failed to produce any neuroprotective effect. However, when dosed in combination, ARL17477 and MK-801 together provided a significantly increased degree (78%) of neuroprotection.

In a subsequent set of experiments we evaluated the effects of LY293558 alone or in combination with either 7-nitroindazole or ARL17477. LY293558 20mg/kg was administered by the i.p route 30 minutes prior to occlusion followed by two further treatments at 3 hourly intervals, where the dose was reduced to 10mg/kg. As previously, 7-nitroindazole 25mg/kg i.p and ARL17477 25mg/kg i.p were administered immediately and again 3 hours post occlusion. Firstly, LY293558 provided significant neuroprotection (20%) and 7-nitroindazole produced a small (10%) degree of protection, which failed to reach significance. However, when dosed in combination, LY293558 and 7-nitroindazole provided a significantly increased (44.5%) neuroprotection. In the second study, LY293558 provided more marked degree of neuroprotection (37%) when dosed alone, while ARL17477 showed no effect, as seen previously. However, when LY293558 and ARL17477 were dosed in combination the degree of neuroprotection observed was significantly greater (71%) than the calculated additive effects of the individual treatments.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood by one of the ordinary skill in the art, that the practice of the invention encompasses all of the usual variations, adaptations, or modifications, as come within the scope of the following claims and its equivalents.

WHAT IS CLAIMED IS:

1. A method of treating a neurological disorder comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

2. The method according to claim 1 wherein the neurological disorder is global cerebral ischaemia.

3. The method according to claim 1 wherein the neurological disorder is focal cerebral ischaemia.

4. The method according to claim 1 wherein the nitric oxide synthase inhibitor is selected from the group consisting of 7-nitroindazole, ARL 17477, 3-Bromo-7-nitroindazole, L-MIN, L-NAME, and 1-(2-trifluoromethylphenyl) imidazole.

5. The method according to claim 4 wherein the nitric oxide synthase inhibitor is 7-nitroindazole or ARL 17477.

6. A method according to claim 1, wherein the excitatory amino receptor modulator is an excitatory amino receptor antagonist.

7. The method according to claim 6 wherein the excitatory amino receptor antagonist is an AMPA receptor antagonist.

8. The method according to claim 6 wherein the excitatory amino receptor antagonist is an NMDA receptor antagonist.

9. The method according to claim 6 wherein the excitatory amino acid receptor antagonist is a kainate receptor antagonist.

10. The method according to claim 1 wherein the excitatory amino acid modulator is an mGluR2 or mGluR3 agonist.

11. The method according to claim 6 wherein the excitatory amino acid modulator is an mGluR1 or mGluR5 antagonist.

12. The method as claimed in claim 7 wherein the AMPA receptor antagonist is selected from the group consisting of NBQX, GKYI 52466, LY293558, LY300164, LY300168, YM90-K, and YM872.

13. The method as claimed in claim 12 wherein the AMPA receptor antagonist is LY293558 or LY300164.

14. The method as claimed in claim 8 wherein the NMDA receptor antagonist is MK-801, CNS1102, Aptiganel, Ro-01-6794/706, Dextromethorphan, NPS1506, memantine, D-(–)CPP-ene, CGS 19755, LY233053, LY202157, Ramacemide, HA-966, ACEA1021, GV150526A, L-701273, ifenprodil, and eliprodil.

15. The method as claimed in claim 14 wherein the NMDA receptor antagonist is MK-801.

16 . The method as claimed in claim 9 wherein the kainate receptor antagonist is LY377770 or LY382884.

17. The method as claimed in claim 10 wherein the mGluR2 or mGluR3 agonist is LY354740, LY379268 or LY389795.

18. A pharmaceutical composition comprising a nitric oxide synthase inhibitor and an excitatory amino modulator, in combination with one or more pharmaceutically acceptable carriers, diluents, or excipients.

19. The use of an excitatory amino acid receptor modulator in combination with a nitric oxide synthase inhibitor for the manufacture of a medicament for treating a neurological disorder.

20. A method of treating a neurological disorder or a neurodegenerative disease comprising administering to a patient an effective amount of a compound which possesses the combined activities of a nitric oxide synthase inhibitor and an excitatory amino receptor modulator.

21. A pharmaceutical composition comprising a compound which possesses the combined activities of a nitric oxide synthase inhibitor and an excitatory amino modulator, in combination with one or more pharmaceutically acceptable carriers, diluents, or excipients.

22. A method of treating a neurodegenerative disease comprising administering to a patient an effective amount of a nitric oxide synthase inhibitor in combination with an effective amount of an excitatory amino receptor modulator.

23. A method of potentiating the neuroprotective effect of an excitatory amino acid receptor modulator in a patient, comprising administering to said patient an effective amount of a nitric oxide inhibitor.

24. The use of a nitric oxide synthase inhibitor for the manufacture of a medicament for potentiating the neuroprotective effect of an excitatory amino acid receptor modulator.

25. A method of potentiating the neuroprotective effect of a nitric oxide inhibitor in a patient, comprising administering to said patient an effective amount of an excitatory amino acid receptor modulator.

26. The use of an excitatory amino acid receptor modulator for the manufacture of a medicament for potentiating the neuroprotective effect of a nitric oxide synthase inhibitor.